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Assembly of hydrogel units for 3D microenvironment in a poly(dimethylsiloxane) channel

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Abstract

Construction of three-dimensional (3D) microenvironment become an important issue in recent biological studies due to their biological relevance compared to conventional two-dimensional (2D) microenvironment. Various fabrication techniques have been employed to construct a 3D microenvironment, however, it is difficult to fully satisfy the biological and mechanical properties required for the 3D cell culture system, such as heterogeneous tissue structures generated from the functional differences or diseases. We propose here an assembly method for facile construction of 3D microenvironment in a poly(dimethylsiloxane) (PDMS) channel using hydrogel units. The high-aspect-ratio of hydrogel units was achieved by fabricating these units using a 2D mold. With this approach, 3D heterogeneous hydrogel units were produced and assembled in a PDMS channel by structural hookup. In vivo-like 3D heterogeneous microenvironment in a precisely controllable fluidic system was also demonstrated using a controlled assembly of different types of hydrogel units, which was difficult to obtain from previous methods. By regulating the flow condition, the mechanical stability of the assembled hydrogel units was verified by the flow-induced deformation of hydrogel units. In addition, in vivo-like cell culture environment was demonstrated using an assembly of cell-coated hydrogel units in the fluidic channel. Based on these features, our method expects to provide a beneficial tool for the 3D cell culture module and biomimetic engineering.

Background

Three-dimensional (3D) environment in a microfluidic channel has received much attention in both biology and bioengineering field since the demand for mimicking an in vivo like nature condition on a miniaturized system increases [1]. In contrast with standard flat cell culture, microfluidics-based cell culture systems provide advantages such as reduced sample and reagent consumption, precise control of culture environment and automated operation. Such small-sized integrated systems are also suitable for in situ analysis of cells, including real-time assay, monitoring, and measurement, which are crucial for the micro total analysis systems (μTAS). Furthermore, an artificial 3D environment can be used to improve the physiological relevance of cell or tissue and to reduce the gap between the in vitro system and the in vivo state, which emphasizes the importance of 3D environment [2, 3]. These features overcome the limitations of conventional in vitro cell culture system and facilitate lots of applications such as in vitro biological study [4–6], drug development [7–9] and cancer research [10–12].

To date, various methods of microfabrication techniques have been applied to construct a microfluidic 3D environment based on the purpose, design and function of its device. Most of the researches were performed by geometrically trapped cells using pillars [13, 14], microwell [15], chamber [16, 17], use of structural guidance using scaffold made of natural and/or synthetic materials [18–21], and microchannel itself made of biocompatible materials [22]. Each of these techniques is available to construct a simple microfluidic 3D environment, however, it is difficult to obtain multiple criteria such as

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complex geometry, mechanical property, biocompatibility, usability and durability, which are crucial to the cell culture environment in a microfluidic system. Although the trapped cells can be easily applied for the facile supply and retrieval of media, reagents, and other chemicals, the lack of extracellular matrix that is indispensable for binding, interaction, and proliferation of cells causes a different behavior of the cultured cell compared with in vivo state. Meanwhile, complex multi-step fabrication processes are generally required to construct 3D structures based on scaffold made of natural/synthetic hydrogel. In addition, structures and functions of hydrogel scaffolds are limited due to intrinsic characteristics of hydrogel materials. To obtain better mechanical properties, there is a certain loss of biocompatibility, and vice versa. Thus, a new method is needed to develop a new model for the construction of a 3D environment to overcome these limitations.

Here, we present a novel method for the construction of a 3D environment by assembling 3D hydrogel units in a poly(dimethylsiloxane) (PDMS) channel. 3D hydrogel units were fabricated by a replica molding process using a hydrogel precursor and a PDMS mold. In general, a high-aspect-ratio structure has been formed using a complex fabrication process based on the z axis 3D structure. In contrast, we designed our high-aspect-ratio structures on a two-dimensional (2D) plane. After fabricating the planar structures, we formed 3D structures, whereby the planar structures stand vertically by geometrical fitting in a fluidic channel. In this study, we have mimicked villi structure of intestine as an example of high-aspect-ratio structures. 3D heterogeneous microenvironment was achieved via consecutive assembly of different hydrogel units in a fluidic channel. We also investigated the mechanical durability of hydrogel units under the flow-induced mechanical stimulus in the hydrogel unit-assembled PDMS channel. Finally, we demonstrated in vivo-like 3D environment by assembling cell-coated hydrogel units in the fluidic channel.

Methods

Design of hydrogel unit and fluidic channel

The purpose of our platform is to construct an on-chip controllable 3D cell culture environment using a cell-culturable hydrogel and a supporting polymer channel. For the 3D cell culture environment under flow conditions, we developed an assembly method in which 3D hydrogel units can be geometrically intermeshed with a polymer channel device. In this study, we have mimicked a human intestinal microenvironment that has complex 3D structures under controlled flow conditions. As shown in Fig. 1, a hydrogel unit is a basic unit to be assembled on a PDMS fluidic channel.

The hydrogel unit consists of three parts: basal part, villi and latch blocks (Fig. 1a). The basal part is a long rectangular block, which plays a role as a structural backbone of hydrogel unit. The width (x), length (y) and height (z) of the basal part are 150 μm × 4150 μm × 150 μm. Latch blocks at both ends of the basal part are protruded perpendicular to the basal part and villi structures. The dimensions of the latch block are 150 μm × 300 μm × 150 μm. Villi are protruded from the basal part and become narrow toward the rounded-end of the villi. On the basis of the dimension of the real human intestinal villi [23], the width and length of the villi structures are designed to be 150 μm, the heights are 300, 450, 650 and 1000 μm, and the gap between villi is 150 μm.

The fluidic channel is designed to be integrated with a hydrogel unit and used to build up a controllable fluidic condition (Fig. 1b). The fluidic channel consists of two parts: fluidic chamber and latch structure. The fluidic chamber (12,150 μm × 3150 μm × 1150 μm) is the place where hydrogel units have been assembled and the fluid flow. Latch structures are located along the fluidic chamber at both sides as the shape of the long groove and the narrow slots. The long groove (3150 μm × 300 μm × 130 μm) and the narrow slots (150 μm × 300 μm × 130 μm) of the latch structure are geometrically fitted with latch blocks of the hydrogel unit and prevent disassembly after assembly.

Fabrication of hydrogel unit mold and fluidic channel

We used conventional photolithography and PDMS replica molding method to fabricate a hydrogel unit mold and a PDMS fluidic channel. To fabricate protruded latch blocks on the basal part, we performed two-step photolithography. Compared to the molds used in other 3D structure studies, we exploited a conventional fabrication process to make high-aspect-ratio structures by patterning on a 2D plane. This method makes it to produce various high-aspect-ratio structures by a simple photolithography process. Briefly, the first layer of negative photoresist SU-8 2100 (MicroChem Corp., MA, USA) was coated on a 4-inch Si wafer with 150 μm thickness. The basal part and villi structures were patterned on a 2D plane (y–z plane shown in Fig. 1) using photomask and UV light. The second layer of the same photoresist was coated on the first layer with 150 μm thickness and latch blocks were patterned. After developing both layers, a mixture of PDMS (Dow Corning, Midland, MI, USA) and curing agent (10:1) was poured onto patterned Si wafer, cured on a hot plate, and peeled off after curing.

The fabrication of a fluidic channel was performed in a similar way to the fabrication of hydrogel mold unit, however, several more steps were needed to make a deep fluidic chamber. Latch structures and fluidic chamber

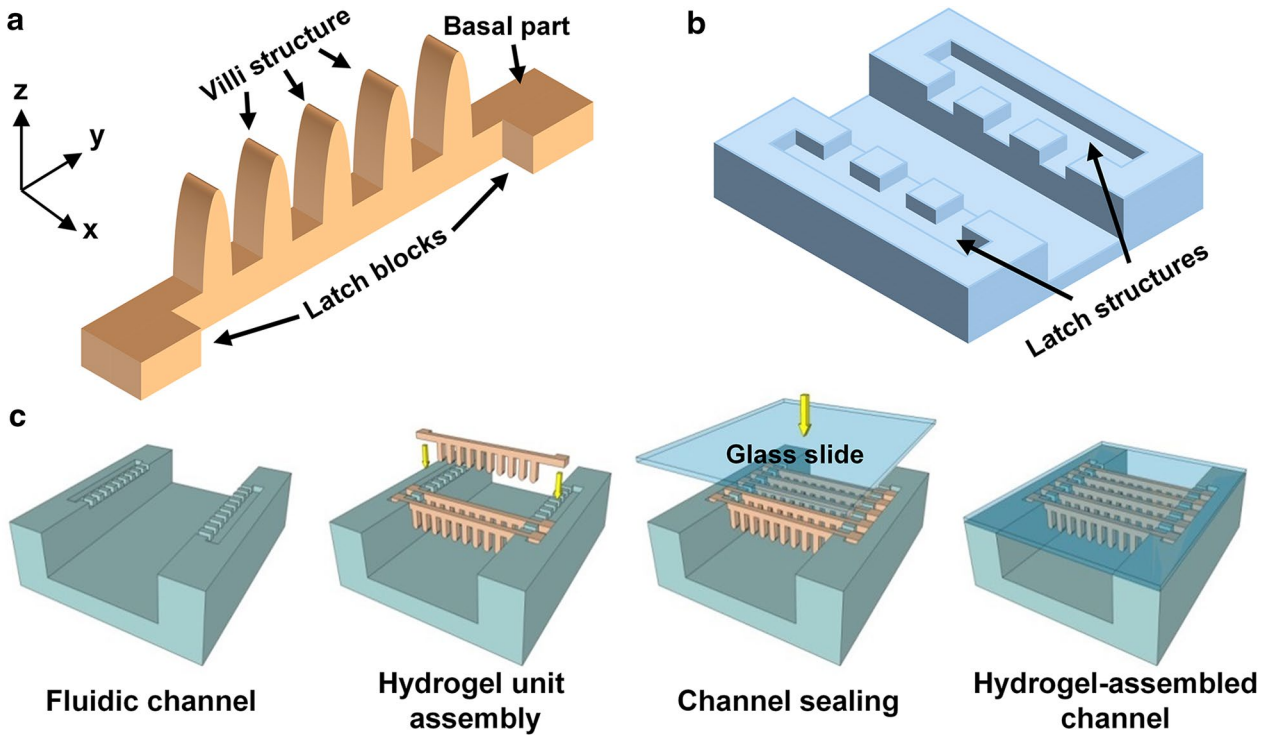

Fig. 1 Design of hydrogel unit, fluidic channel and assembly process. **a** A hydrogel unit consists of basal part, villi structure, and latch blocks. **b** Fluidic channel has latch structures where the latch blocks of hydrogel units are geometrically locked. **c** Schematic representation of the assembly process. Hydrogel units are assembled in a fluidic channel by intermeshing latch blocks and latch structures. After assembly, the fluidic channel is covered with a glass slide to seal the microfluidic environment

were patterned on a 4-inch Si wafer with 130 μm thickness. A mixture of PDMS and curing agent (10:1) was poured onto patterned Si wafer with 1.5 mm thickness, cured on a hot plate, and peeled off after curing. After that, the ceiling of the fluidic chamber was cut off to make an open chamber with 1.5 mm height, while the height of the latch structures was 130 μm. Then, a flat PDMS plate was bonded on the opposite side of the latch structures to make a ceiling of the fluidic chamber.

Fabrication of hydrogel units

In consideration of structural and functional properties of hydrogel materials, we selected alginic acid sodium salt (Alginate; Alfa Aesar, Ward Hill, MA, USA) and collagen type I from rat tail (BD Bioscience, Franklin Lakes, NJ, USA). Rigid mechanical properties of alginate are suitable for the structural backbone of hydrogel units and collagen ensures enhanced cell adhesion and proliferation. To satisfy these properties, we set the final concentration of alginate as 1.5 wt% dissolved in phosphate buffered saline (PBS; Thermo Fisher Scientific, Waltham, MA, USA) and collagen as 3 mg/mL neutralized with 0.1 N NaOH. Prior to loading prepared hydrogel mixture to the mold, the surface of the PDMS mold was coated with 3 wt% Pluronic F-127 (Sigma) solution for 2 h to avoid adhesion of the hydrogel to the mold, followed by mild washing with distilled water. The hydrogel mixture was loaded to a surface-treated PDMS mold using a pipette or a syringe. The hydrogel-loaded PDMS mold was incubated in 37 °C, humidified condition for 1 h to crosslink collagen. Then, 100 mM $CaCl_2$ solution dissolved in distilled water was nebulized on the mold for 2 min to crosslink alginate. Fully crosslinked hydrogel units were peeled off from the PDMS mold by mild pipetting. For the hydrogel visualization, 50 mM methylene blue solution was used.

Cell culture and cell coating on hydrogel units

Human epithelial colorectal adenocarcinoma cells (Caco-2) were grown in minimum essential media (MEM; Thermo Fisher Scientific) supplemented with 20% fetal bovine serum (FBS; Thermo Fisher Scientific) and 1% penicillin–streptomycin (Thermo Fisher Scientific). Cell cultures were maintained for 7 days on a conventional 100 mm petri dish at 37 °C in a 5% CO_2 and 95% air humidified atmosphere. The culture media was replaced after 1 day of seeding and every 2 days in a culture period.

To prepare the cell coating on a hydrogel unit, the cells were detached and concentrated to enhance the efficiency

of cell coating. To detach the cells from the dish, remove the media from the dish and wash with 10 mL of PBS. Then, 1 mL of 0.05% trypsin–EDTA (Thermo Fisher Scientific) was treated and incubated in cell culture incubator for 3 min. The detached cells were collected with 10 mL of media, centrifuged to collect cells in the bottom of the tube and to remove the supernatant media and trypsin–EDTA. The centrifuged cells were re-suspended with a small volume of media, the concentration of the cell being 5×10^6 cells/mL. This concentrated suspension of Caco-2 cells was incubated with prepared hydrogel units in a microtube overnight inside a cell culture incubator.

Assembly process of hydrogel units in a PDMS fluidic channel

The assembly process of hydrogel units in a fluidic channel was carried out by intermeshing of the latch structure and the latch block. A schematic of the assembly process is shown in Fig. 1c. First, the hydrogel unit is aligned perpendicular to the direction of the fluidic channel. Then the latch blocks at both ends are fitted to groove and slot structures. The assembled latch blocks in the groove are structurally fixed by slot structures. This geometrical locking between the fluidic channel and the hydrogel unit makes a structural support inside a channel and maintains its condition against the flow.

The fluidic channel was plasma treated for 30 s in a plasma cleaner (FEMTO Science Inc., Yongin-si, Korea) to make a hydrophilic surface to prevent bubble formation inside a channel after assembly. The plasma-treated channel was filled with PBS, and then fabricated hydrogel units were assembled by being adjusted to enter into grooves and bridge structures. The spacing between the hydrogel units were controlled by the gap in the bridge structures. After the assembly process, the channel was covered with a glass slide and tightly sealed by weight blocks on an assembly jig.

Results and discussion

Fabrication of hydrogel unit

To fabricate hydrogel villi structures using a hydrogel mixture, we used a replica molding technique without complicated or expensive fabrication processes. Several PDMS molds with laterally patterned villi structure were used to fabricate hydrogel villi structures having a long villi length with various aspect ratios. It is difficult to observe hydrogel villi structures under the bright field imaging using a conventional microscope due to its bright color and transparency. Consequently, a dark field imaging mode of stereoscope (SZX2-ILLD; Olympus, Japan) was used to observe hydrogel villi structures. To demonstrate an easy fabrication for size-controllable hydrogel villi, we fabricated several types of hydrogel villus of different dimensions. In vivo intestinal villus has various length range from 300 to 1000 μm, we chose four length types of the villi structure; 300, 450, 650 and 1000 μm and their aspect ratios were 2.0, 3.0, 4.3, and 6.8, respectively. As shown in Fig. 2, we have verified that hydrogel villi were successfully fabricated regardless of their aspect ratios. This suggests that a conventional fabrication process for high-aspect-ratio structures would be circumvented by the simple fabrication process.

The shrinking phenomenon of hydrogel scaffold was observed just after the nebulization of $CaCl_2$ for alginate crosslinking. It showed a reduction of 5–10% compared to the hydrogel villi and the PDMS mold. After the mold was immersed in the media, however, the reduced hydrogel villi returned to their original size. A few minutes after the media supply, the hydrogel villi were tightly fitted to the mold. It seems that these phenomena resulted from the movement of water across hydrogel villi structure. In this work, hydrogel villi structures were fabricated by an alginate–collagen mixture and the gelation of alginate was based on electrostatic crosslinking by calcium ion. Accordingly, the difference in the ion concentration between the hydrogel and the surrounding media causes an ion concentration gradient which induces the movement of water. It seems that this movement of water causes swelling and shrinkage of the hydrogel villi structure.

Controlled assembly of hydrogel units for heterogeneous condition

Each hydrogel unit was assembled in parallel to form a hydrogel villi array structure inside a fluidic chamber (Fig. 3a). Methylene blue solution was used to aid the visualization of hydrogel units. Each hydrogel unit was upright in the chamber and its shape remained stable under the flow in the chamber. The bottom view of the assembly shows that the latch structures of hydrogel units and fluidic channel were firmly anchored (Fig. 3b).

Meanwhile, the ability to build a 3D hydrogel environment with controllable dimensions is one of the challenging issues in bioengineering field. Conventionally, a 3D hydrogel environment has been obtained to structurally mimic the in vivo state by exploiting an array type of microstructure so that all the structures are formed on the same dimensions. However, these homogeneous environmental conditions are not suitable for simulating heterogeneous in vivo conditions. To build up a controllable 3D environment, various types of hydrogel units were assembled according to their dimensions, ingredients and materials to demonstrate the ability to construct heterogeneous environmental conditions in the same system. Hydrogel units of four different dimensions were assembled in a fluidic channel in the order of height (Fig. 3c). As shown in Fig. 3d, the assembled hydrogel

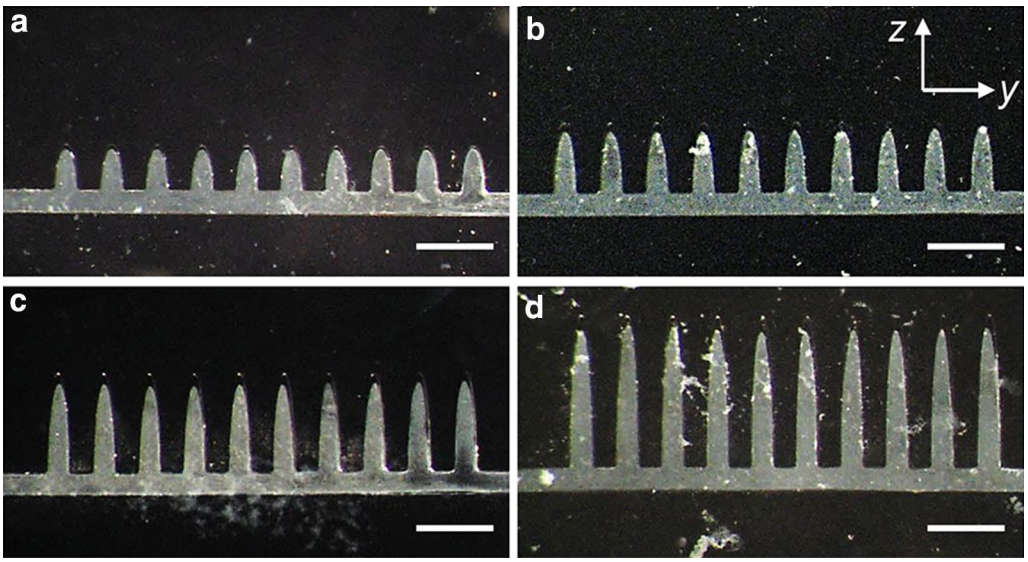

Fig. 2 Various size of the hydrogel villi structure on the PDMS mold. We verified that hydrogel villi were successfully fabricated irrespective of their long dimension and aspect ratio, which was limited by conventional methods. The height of the villi structure was **a** 300 **b** 450, **c** 650, and **d** 1000 μm. *Scale bars* 500 μm

units were well formed in a fluidic channel to make a heterogeneous condition. We have also verified that they have been kept stable in a channel under flow conditions.

To simulate the capability of controllable environment of our platform, we assembled different types of hydrogel units. We fabricated two types of hydrogel units containing different colored fluorescent beads as control

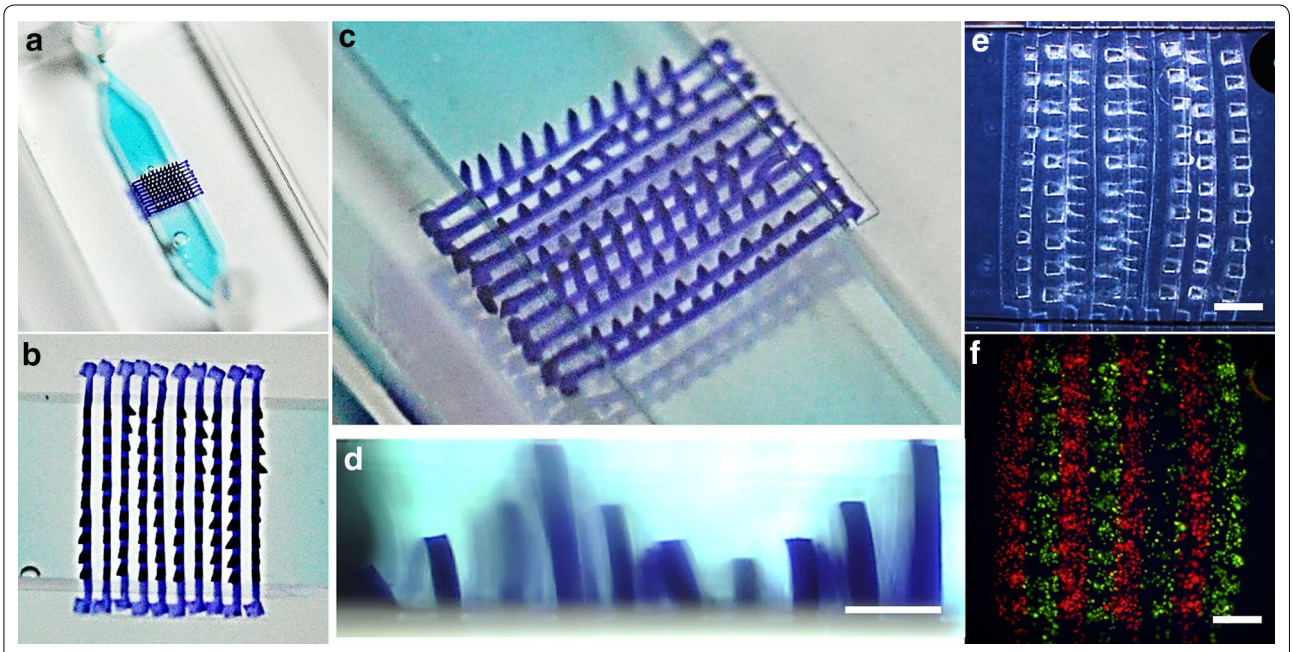

Fig. 3 Hydrogel units assembled with a fluidic channel. **a** Hydrogel units were arranged to form a villi array structure in a fluidic chamber. **b** Latch structures of the hydrogel unit were intermeshed with a groove of the fluidic channel. **c** Hydrogel units with four different heights were arranged in a fluidic chamber to form heterogeneous microenvironments. **d** A side view of the heterogeneous assembly shown in **c**. Different lengths of hydrogel units were arranged as a mountain chain. *Scale bars* 500 μm. Methylene blue solution was used to aid visualization by *blue color*. **e**, **f** Hydrogel units containing different fluorescent microbead are alternately assembled for the content-controlled heterogeneous microenvironment. **e** Bright field image shows stably assembled hydrogel units, and **f** fluorescent image shows *red* and *green color* appeared alternately. *Scale bars* 500 μm

material. During the fabrication of hydrogel units, red and green fluorescent beads of 10 μm diameter were added to uncrosslinked hydrogel mixture. These two types of hydrogel villi were alternately assembled in a channel. The bright field image (Fig. 3e) showed the hydrogel units assembled in a single fluidic channel, and the red and green fluorescent signals appeared alternately in the channel (Fig. 3f). The controllable assembly of our platform provides us various capabilities of changing microenvironment, including dimensions, contents inside hydrogel units, and even material of each hydrogel unit in the same system. This controllability would be benefit to construct a complex in vivo-like environment with various types of cells or supporting materials.

Flow-induced deformation of villi structures

We have already verified the stability of the assembled hydrogel units in a fluidic channel. However, due to the deformability of the soft hydrogel, the assembled hydrogel units showed a flexible deformation under flow conditions, and were recovered after the flow stopped. To characterize the relation between the applied flow rate and the deformation, we performed deformation tests: 300, 450, 650 and 1000 μm height of the hydrogel villi were tested under varying flow velocity. In the case of small intestine, food and liquid flow at a speed of 1 m/h on average [24], this is equivalent to about 50 μL/min in our fluidic channel. Based on this information, various flow rates of cell culture media ranging from 30 to 200 μL/min were applied into the channel, while the deformation of hydrogel villi was observed. Figure 4 showed the deformation of 650 μm hydrogel villi under 50, 100, and 200 μL/min, respectively. We measured the displacement change ($D-D_0$) of a tip of assembled hydrogel units under applied flow using a microscope and a CCD camera (Fig. 4a). Hydrogel units were imaged on the top side of the channel, and the displacement change was calculated using the imageJ program. Although the displacement of the tip of the hydrogel villi was not a direct measurement of the deformation, the trace of hydrogel villi was proportional to the length and deformation angle of the hydrogel villi.

As shown in Fig. 4b, the deformation of the hydrogel villi increased as the flow rate increased under the same length. Additionally, the deformation increases as the length of hydrogel villi increases under the same flow rate. From these observations, we concluded that the deformation of the hydrogel villi is related to the length of the hydrogel villi as well as to the flow rate. Based on this relationship, we could construct a 3D cell culture environment with controlled deformation of hydrogel villi that is critically related to the shear force applied to the cultured cells on the hydrogel surface. We expect various conditions to be modeled using different types and ratios of hydrogel materials on our platform.

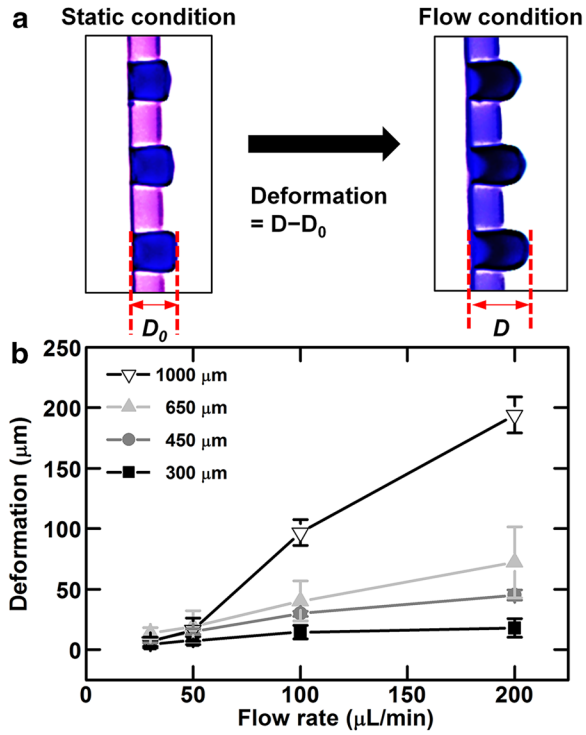

Fig. 4 Deformation of villi structures under flow condition. **a** Villi structures of the hydrogel unit (height = 650 μm) under static and 200 μL/min conditions, respectively. Deformation was calculated by measuring the difference of the lateral position of the tip of the villi structures. **b** Flow-induced deformations of four different dimensions of hydrogel villi were measured under various flow rate conditions. The deformation of the hydrogel villi increased as the applied flow rate and the height of hydrogel villi increased

Cell-coated hydrogel units assembled in a fluidic channel

Due to the available binding sites of hydrogel materials, it is expected that our hydrogel unit is appropriate for cell culture on its surface. In this study, we coated Caco-2 cell on the surface of the hydrogel units to mimic intestinal microenvironment. As shown in Fig. 5a and b, Caco-2 cells were attached on the surface of villi structures and basal part of hydrogel units. It was reported that the bottom places between villi structures were a biological niche where various intestinal cell were differentiated and proliferated into villi structures [25]. From the result, it is expected that our hydrogel unit could be exploited to observe cell proliferation and migration on the surface.

To verify a 3D cell culture environment on a chip under flow conditions, cell-coated hydrogel units were assembled in a fluidic channel. During the entire 2 day-cell culture on a chip, it was confirmed that the assembled hydrogel unit and the attached cells were kept stably

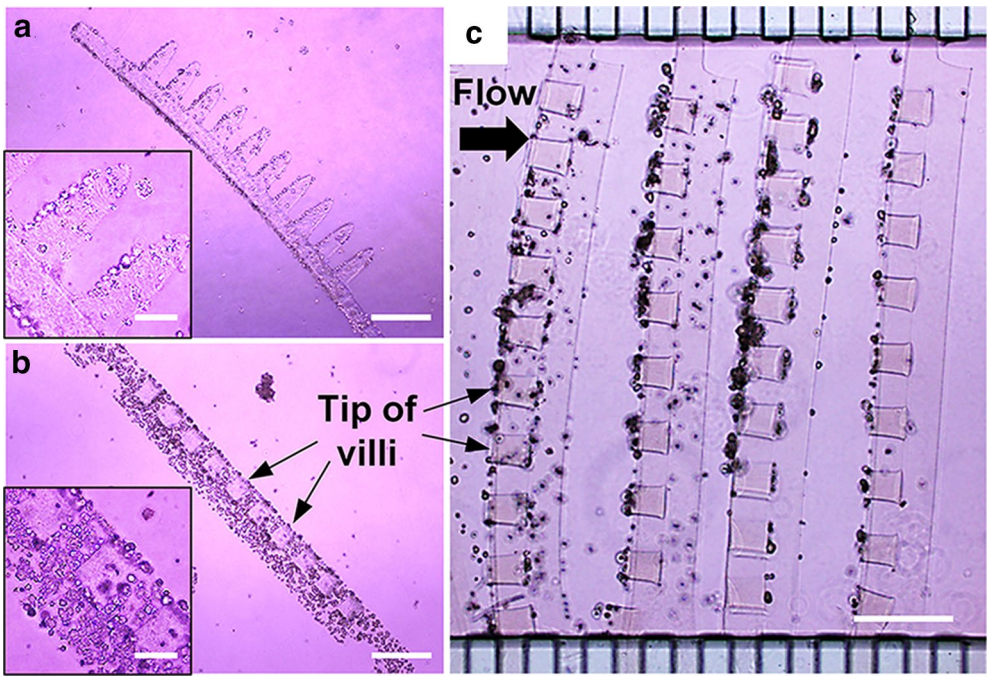

Fig. 5 Cell-coated hydrogel units and assembly with channel. **a**, **b** *Side view* (**a**) and *top view* (**b**) of a cell-coated hydrogel unit. *Insets* show the magnified view of the hydrogel unit and we verified that Caco-2 cells were well attached on the surface of villi structures and basal part. **c** *Top view* of cell-coated hydrogel units assembled in a fluidic channel. In vivo-like 3D cell culture environment under controllable fluidic conditions has been developed. *Scale bars* 500 and 100 μm for inset

under the flow of cell culture media (Fig. 5c). Although some swelling or shrinkage (up to 10%) was observed, no remarkable signs of fracture or denaturation were found. Even with a sudden change in the flow rate (0–200 μL/min), the hydrogel units smoothly respond to the changes in the flow rate without any fracture. By regulating the flow rate and nutrients of culture media, which are essential for relevant in vivo-like modeling, various cell culture conditions were also obtained (data not shown). On the basis of these observations, our cell-coated hydrogel unit will be a suitable tool for studying intestinal microenvironment.

Conclusions

In this study, we introduced a novel method to construct a 3D environment in a PDMS fluidic channel by assembling heterogeneous hydrogel units. By exploiting assembled hydrogel units, we were able to easily obtain high-aspect-ratio structures made of biocompatible hydrogel materials which are suitable for a controllable microfluidic environment. The assembly method showed a versatility in the use and arrangement of hydrogel units. It is expected that controllable deformation of the assembled hydrogel unit in a fluidic channel will extend the potential applications of the assembled hydrogel units to mechanobiology. In addition, an assembly process of cell-coated hydrogel units can be provided simple construction of a 3D cell culture environment under fluidic conditions. By simply changing the structure and/or material of hydrogel units, we can create a heterogeneous cell culture environment suitable for a target cell, resulting in biologically relevant results as compared to the conventional platform. Furthermore, the assembled hydrogel units can be easily retrieved by disassembling the fluidic channel after the experiments, thereby facilitating further post-analysis using the cultured assembled hydrogel units. Based on these strengths, our method could provide a wide range of applications such as in vitro 3D cell culture module and biomimetic engineering studies.

Authors' contributions

CHC and JKP designed research. CHC carried out experiments. CHC, SK, and JKP analyzed the data. CHC and JKP wrote the paper. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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